UNIVERSITY OF GONDAR

COLLEGE OF MEDICINE AND HEALTH SCIENCES

DEPARTMENT OFINTERNAL MEDICINE

FACTORS ASSOCIATED WITH HIV VIROLOGIC TREATMENT FAILURE AMONG HIV INFECTED CLIENTS ON HIGHLY ACTIVE ANTI-RETROVIRAL THERAPY AT GONDAR UNIVERSITY HOSPITAL, NORTHWEST ETHIOPIA, 2015.

BY. Belete Bayu (BSC)

Advisors. Dr. Abera Balcha (MD, Internist)
Mr. Amare Tariku (BSC, MSC)

A THESIS TO BE SUBMITTED TO THE DEPARTMENT OF INTERNAL MEDICINE, COLLEGE OF MEDICINE AND HEATLH SCIENCES, UNIVERSITY OF GONDAR,IN THE PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER'S OF SCIENCE IN CLINICAL TROPICAL INFECTIOUS DISEASE AND HIV MEDICINE.

June, 2015
Gondar, Ethiopia

UNIVERSITY OF GONDAR

COLLEGE OF MEDICINE AND HEALTH SCIENCES

DEPARTMENT OFINTERNAL MEDICINE

Factors associated with HIV Virologic treatment Failure among HIV infected clients on Highly Active Anti-retroviral Therapy at Gondar University Hospital, Northwest Ethiopia, 2015.

BY Belete Bayu

Mobile----------

Email----------

Approved by the examining bored

Director school of medicine

Advisor

1. ----------

2. ----------

Examiner

Acknowledgements

My deepest gratitude goes to my advisors Mr. Amare Tariku and Dr..Abera Balcha for their invaluable commitment, support and guidance during the overall process of this proposal development. The last but not the least appreciation will be for the Department of internal medicine , College of Medicine and Health Sciences because the final result of this proposal would be impossible without the continuous support and opportunity given by the this department.

Acronyms and/or Abbreviations

HIV	- Human Immune Virus
AIDS	-Acquired Immune Deficiency Syndrome
VL	-Viral Load
ART	-Anti-Retroviral Therapy
HAART	- Highly Active Anti-Retroviral Therapy
WHO	-World Health Organization
CD4-	Cluster of Differentiation
ARV	- Anti-Retroviral
HIVDR	-Human Immune Virus Drug Resistance

Contents

List of Tables

Abstract

Background: The identification and management of antiretroviral therapy failure is a key challenge for HIV/AIDS programs in resource-limited settings. In Ethiopia diagnosing treatment failure and monitoring patient response with routine viral load, is not feasible. Recently, it has been started targeted viral load monitoring to confirm treatment failure in hospital settings. However factors lead to treatment failure is not well understood and well-studied.

Objective: To identify determinants of antiretroviral virologic treatment failure in Gondar University Hospital.

Methods: An unmatched case control study was conducted at Gondar University Hospital using record review. Total sample size was 306 (153 cases and 153 controls). The study was done from May to June. Bivariate analysis was done and all explanatory variables associated with treatment failure with P<.0.2 were entered in to multivariable logistic regression analysis using back ward stepwise likelihood ratio method to identify independent predictors to identify independent predictors.

Result: One hundred fifty three cases and One hundred fifty three controls were included in the study. Poor adherence (Adjusted odds ratio (AOR), 29.0, 95% confidence interval (CI), 11.3 - 75.4), recent clusters of differentiation cell count (CD4<200 cells/µl (AOR, 13.8, 95% CI, 5.7 - 33), age (25-34)(AOR, 8.8, 95%, CI, 2-31.) and unmarried(AOR, .33, 95% CI, .12 - .87) were all independently associated with antiretroviral virologic treatment failure.

Conclusion and Recommendation: The independent associated factors for antiretroviral virologic treatment failure were; recent cd4 cell count lower than 200 cell/µl, history of poor adherence for ART, age<34years and unmarried. There for Health professionals should pay special attention for the risk group identified.

Keywords: antiretroviral therapy, case control study, treatment failure, highly active Anti-Retro-Viral.

1. Introduction

1.1Statement of the problem

The impact of HIV infection has dramatically been altered with the introduction of antiretroviral drugs in 1987 and modification by combination treatment, known as highly active antiretroviral therapy (HAART), in 1996. After three years following the introduction of HAART, mortality, AIDS-defining diagnoses, and hospitalizations all decreased 60 to 80%(1, 2).

About 20 ARV drugs available of which one injectable and the others are tablets and these are generally classified as fusion inhibitors, nucleoside –reverse transcriptase inhibitors (NRTIs),non-nucleosidereversetranscriptase inhibitors (NNRTIs), Integrate inhibitors, and protease inhibitors (PIs).Resource limited settings use the WHO recommendation 2NRITs + NNRTIs as first line regimen and 2NRITIs+ PIs as second line treatment regimen (1, 3).

Even though ART dramatically changed the impact of HIV infection, eradication of the virus cannot be achieved with available antiretroviral (ARV) regimens even when new, potent drugs are added to a regimen that is already suppressing plasma viral load below the limits of detection of commercially available assays) (1, 4, 5).

Use of ART has been associated with the development of HIV drug resistance (HIVDR). Because of the error- prone nature of HIV reverse transcriptase, the virus‟ high mutation rate in the presence of drug selective pressure, and because of the need for lifelong treatment, it is anticipated that some degree of HIVDR will occur among populations on treatment even when appropriate ART regimens are provided and optimal adherence to therapy is supported(1, 6).

Treatment failure occurs when the anti-HIV medications (HAART) taken can't control HIV RNA replication in the body. Based on the monitoring strategies, treatment failure can be categorized as: virologic failure, immunologic failure, and clinical failure(3, 7, 8).Hence viral load monitoring and follow-up is not feasible in resource limited settings; clinical and immunologic criteria have been used to monitor virologic failure which has low sensitivity and positive predictive. Given that the true magnitude of ART treatment failure was not well known in resource limited settings. In the absence of virologic monitoring; diagnosis of ART treatment failure is difficult and many HIV infected patients are died, delayed to switch to second line treatment and unnecessary switch to coasty second line regimen in resource

limited settings(5). As WHO 2014 consolidated ART guideline definition, Virologic failure is viral load >1000c/ml While taking HAART for ≥ 6 months(7, 8).A rate of treatment failure in WHO-recommended first-line antiretroviral regimens in resource-limited settings was six percent. The failure rate per hundred year of follow-up (P100YFUwas 6.08).In Asia 2.55 and in Africa 7.1(9).

According to HIV Related Estimates and Projections for Ethiopia in 2013, the total number of patients ever started on treatment was 499,412 out of 822,531 patients. Currently, 1.5 % of the patients are on 2nd ART line regimens(8).

At the beginning of ART era the rate of treatment failure was high with single or dual therapy, but in the era of HAART the rate is reduced. But as ART scale-up increases the number of HIV infected clients risk of treatment failure also increases proportionally(1).

In Ethiopia ART treatment monitoring and follow up is based on the WHO clinical and immunological criteria to predict virologic failure, and (recently it has started targeted VL monitoring).Because there is scarcity of information related to Predictors of HIV virologic treatment failure at Gondar university hospital. This study determines the Predictors of virologic treatment failure among adult ART patients at Gondar University Hospital

1.2. Literature Review

Risk factor for viroogic failure

A case-control study designed to know the association of pre-existing Minority Drug-Resistant HIV-1 Variants, Adherence and risk of antiretroviral treatment failure shows that detection of minority Y181C mutants was Associated with an increased risk of virologic failure in the setting of recent treatment adherence but not in non-adherent subjects . In adherent patients, pre-existing minority Y181C mutants more than tripled the risk of virologic failure of first-line efavirenz-based antiretroviral therapy (13).

Another study done at nine research center North and South America the presence of K103N mutation at initiation of therapy was associated with VF in both arms (14).Higher pretreatment or baseline HIV RNA level, lower baseline CD4 count, Co-morbidities (e.g., active substance abuse, psychiatric disease, neuro cognitive deficits), Presence of drug-resistant virus, Prior treatment failure, and incomplete adherence(1, 3).

A study shows that: higher base line HIV RNA level was found to be a risk factor for virologic failure(12, 13).

Comment [U1]: which study?

A study in Cameron shows that failure to achieve cd4 count >100cells/mm3 at 6 month was found to be a risk factor of virologic treatment failure(15).Another study shows immunological failure with was associated with VF(10).

Another risk factor associated with HIV virologic treatment failure is adherence status.it was shown by a study done in yawn die, Cameron, pharmacy refill irregularity was strong predictor and it was found to be a better alternative tool to monitor virologic outcome(15).

Other factors associated with HIV virologic treatment failure were long duration on ART(10, 11),followed in rural follow up centers, those patients who haven't disclose their HIV status, were found to be risk factors of HIV virologic treatment failure (10).

Factors associated with failure to achieve viral suppression at 6-months of second-line ART were: adherence <80% and viral load >100,000 at the time of treatment failure. The study demonstrated favorable virological outcomes of the second-line ART in Georgia. Majority of patients, including IDUs, achieve sustained virological response over 36 month period(16).

Suboptimal virologic potency (1, 12),prior exposure to suboptimal regimens(12), high pill burden and/or dosing frequency, adverse drug-drug interactions with concomitant medications, history of drug substitution(1, 3) history of treatment interruption), pruritic popular eruption(PPE)(10) were the other risk factors for HIV virologic treatment failure as shown by different literatures in the world.

the risk factors of treatment failure are generally associated with patient related factor(such as higher baseline HIV RNA level, lower baseline CD4 count, co-morbidities (e.g., active substance abuse, psychiatric disease, and neuro-cognitive deficits), presence of drug-resistant virus, prior treatment failure, and incomplete adherence(1, 3),age <30 years, long duration on ART(10, 11),followed in rural follow up centers, those patients who haven't disclose their HIV status(10)

,and ARV medication related factors(suboptimal virologic potency(1, 12)Prior exposure to suboptimal regimens(12), high pill burden and/or dosing frequency, adverse drug-drug interactions with concomitant medications(1, 3) were the other risk factors for HIV virologic treatment failure as shown by different literatures globally.

a cross-sectional study to compare factors associated with raised viral load between patients with (''experienced'') and without (''naive'') prior antiretroviral (ARV) exposure at commencement of ART at the clinic Shows that raised viral load was associated with prior ARV experience and complete interruption of current ART*(17).*

In Ethiopia the factors associated with treatment failure was studied based on immunologic and clinical criteria, showing that substance use is 1.6 times the hazard of failure compared to those note using, Patient who were ambulatory or bed redden had about 1.81 times hazard of failure compared with working status (HR, 1.81; 95%CI 1.25-2.60) (12),Baseline CD4 count <)250cell/mm3(P<.028), old age group and higher educational status (P,0.001) were significant predictors of immunological treatment failure.(18).

1.3. Conceptual framework

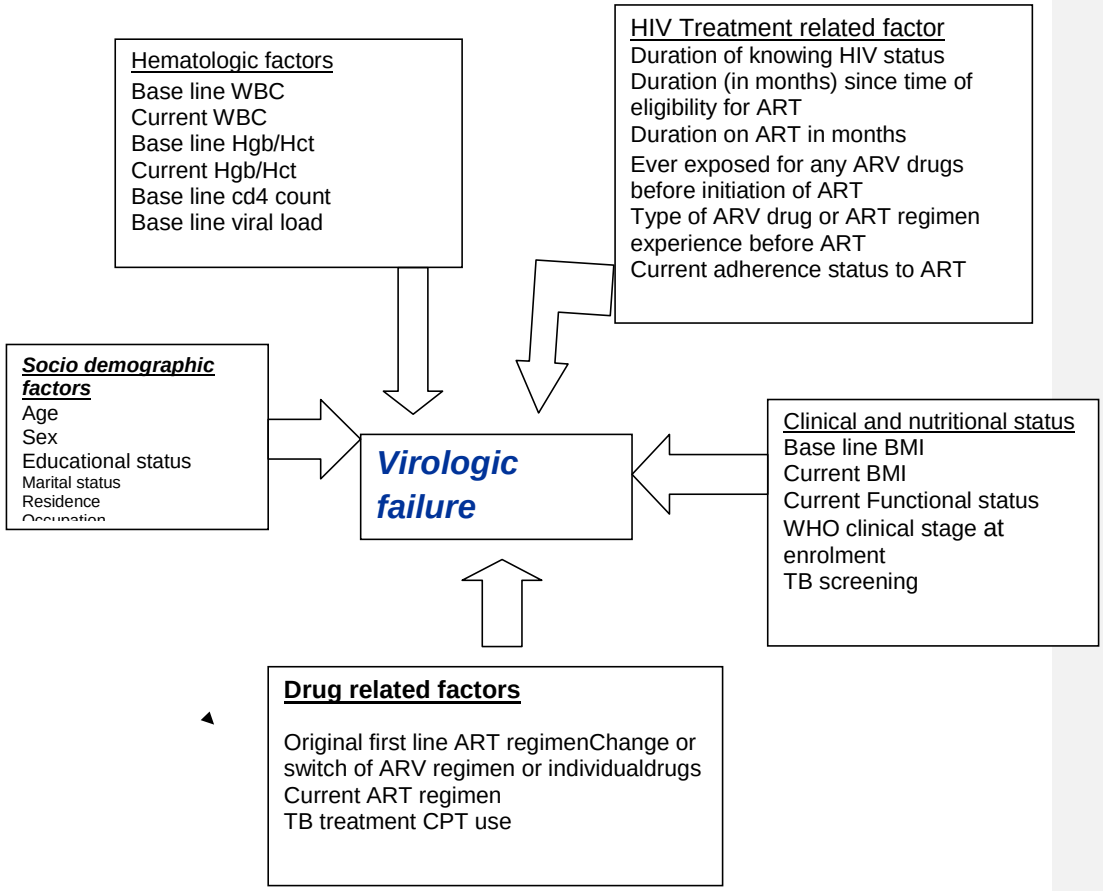

Fig.1 Conceptual framework.

1.4. Justification of the study:

WHO clinical and immunological criteria have been used for monitoring and initiating ART in resource limited settings. But many studies show that it has low sensitivity and positive predictive value to predict virologic failure(19).Because of this, studies shows that rate of switching is below the expected rate which leads to further increment in accumulate drug resistance mutation and unnecessary switching of ART drug regimen. WHO 2013 ART guideline has strongly recommended use of routine virologic monitoring for evaluation and monitoring of ART (7). But till now viral load monitoring is not feasible in low and middle income countries, viral load monitoring has been used for targeted groups (for those who are suspected of treatment failure using WHO clinical and immunologic criteria). Ethiopia is one of the nations which use targeted viral load monitoring starting recently. There is scarcity of evidence on determinates of virologic failure in Ethiopia, therefore this study aims to assess determinants of virologic failure among adult HIV infected client sat Gondar University Hospital, Northwest Ethiopia.

2. Objective

To identify determinants of HIV virologic treatment failure among adult HIV infected clients at Gondar University Hospital, Northwest Ethiopia, 2015.

3. Methods

3.1 Study design and period

Institution-based case-control study was conducted from May June /2015.

3.2 Study area:

The study was conducted in Gondar University Hospitals located in the North West of the country. Gondar University Referral Hospital is found in Gondar Town, 748km far from Addis Ababa to the Northwest of Ethiopia. It is a teaching hospital that acts as a referral center for the nearby general and district hospitals as well as local health centers. Having more than 500 inpatient beds, it gives referral services for about over 5 million inhabitants in the northwest of Ethiopia. The HIV care services of the hospitals were initiated in 2003 and with free ART starts in 2006 and have three clinics: Adult ART clinic, pediatric ART clinic, and VCT clinic. The clinic has one medical doctors providing ART services, two MSc in clinical tropical infectious disease and HIV medicine, one MPH (Master of public health), 1 health officer , 7 BSc nurse ,3 data base manager , 2 porter ,1 janitors ,6 case manager ,8 adherence supporter (people living with HIV). About12,470 HIV infected clients have been enrolled in ART clinic of them 8220 were ever started ART and 5,138 are currently on ART.

3.3 Source of population

All HIV infected adult patients on ART who have followed up at Gondar University Hospital from 2005- May 2015.

3.4 Study population:

All HIV infected adult clients on ART and have follow up at Gondar University Hospital for at least 6month.

3.5 Inclusion criteria and exclusion criteria

3.5.1Inclusion criteria: All HIV infected adult (Age ≥15years) clients on ART who have followed at Gondar University Hospitalfor≥6 month and their viral load test result is documented.

3.5.2 Exclusion criteria: those within complete data (have no intake form, or follow-up chart,)

3.6 Sample size determination and sampling procedure

3.6.1. Sample size determination

Sample size was determined by key predictor using Epi-info -*7.*

Predictor 1 -poor adherence

Predictor 2-low cd4 count at enrolment

No	Key predictors	Assumption	OR	Sample size
1	Adherence	At 95% CI, 80% power.	2.8	146
2	low baseline cd4 count	At 95% CI, 80% power.	7.1	110

Using this predictor's predictor 1 has higher sample size with a total of 146 (73 cases and 73 controls). There were 175 cases and 168 controls. All 175 cases and 168 controls were feasible for this study.

3.6.2. Sampling technique and procedure

Cases: All HIV infected patients with virologic failure (VL>1000C/ml).

Controls: All HIV infected patients without virologic failure (VL<1000C/ml).First those with undetectable VL were selected, then those with lowest detectable VL from controls (VL<1000C/ml) were included to get enough controls.

3.7. Variables of the study

3.7.1. Dependent variables

Virologic failure

3.7.2 Independent variables

Scio-demographic variables

Age

Sex

Residence

Marital status

Level of education

Religion

Occupation

HIV care and anti-retroviral treatment related information

Duration since knowing HIV status

Duration (in months) since time of eligibility for ART

Duration on ART in months

Ever exposed for any ARV drugs before initiation of ART

Type of ARV drug or ART regimen experience before ART

Base line BMI

Current BMI

Current Functional status

WHO clinical stage at enrolment

History of TB disease

CPT use

Adherence status to ART

Original first line ART regimen

Change or switch of ARV regimen or individual drugs

Current ART regimen

Substance use

LABORATORY INFORMATION

Base line WBC

Current WBC

Base line Hgb/Hct

Current Hgb/Hct

Base line cd4 count

Current CD4 count

3.8. Operational definition

Virologic failure (Cases)-viral load> 1000c/ml while on treatment at least six month

Regimen change -its modification of any or all of the initial drugs

Poor adherence - at least one “poor” or “fair” recorded by WHO definition in the follow-up chart.

Good adherence – Good recorded by WHO definition in the follow-up chart.

Substance use- History of grade “++” and above records in the intake form.

Variables expressed as current .to show at the time of viral load test

3.9. Data collection procedures and tools

The data was collected by using a pre-tested, structured questionnaire in English version. Data was extracted from patients chart at ART clinic .Six clinical nurses for data collector and two BSc nurses for supervisor were involved in the study. One day intensive training about objective of the study, data collection or extraction techniques, and variables to be collected was given for data collectors and supervisors. At the beginning and anytime during data collection, sufficient information and clarification was provided for the data collectors by supervisors to come up with quality data.

3.10 Data quality control

The data collected was maintained by using standardized questionnaire and two days onsite training was given to data collectors and supervisors in addition to this ongoing and supportive supervision was implemented throughout the whole time of data collection. Then the collected data was checked for its consistency and completeness before any attempt to enter code and analyze it. Finally, Epi-Info version-7 was used to control and manage errors resulting from data entry process.

3.11 Data processing and analysis

The collected data was cleaned, coded and entered into Epi-Info version 7, and exported to SPSS version20 software for further analysis. Descriptive statistics was used to illustrate the means, standard deviations, medians, ratios, rates, proportions and frequencies of the study variables. Binary Logistic Regression was fitted. Bivariate analysis was run for each independent variable to check the association with virologic failure. Multivariate analysis was also considered so as to control for possible effect of confounders, adjusted odds ratio

with 95% confidence interval was used to determine the strength of association. Variables with p-value ≤ 0.05 were considered as statistically significant to the dependent variable**.** Hosmer and Leme show goodness of fit-test was determined (.066) and it was fit.

4. Ethical consideration

The ethical clearance was obtained from Institutional Ethical Review board of University of Gondar. Letter of cooperation was secured from administrations of Gondar university Hospital. After the permission obtained from ART clinic focal person, data collection process was started. The confidentiality of patient and health care provider related data was maintained by avoiding possible identifiers such as name of the patient, prescriber; only numerical identification was used as a reference then after the whole data collection process, the questionnaire was kept safe throughout the whole process of the research work**.**

5. Result and discussion

5.1. Socio-demographic characteristics of study population

From 363 HIV infected patients data was retrieved from 306 clients' chart. The others were not included due to incomplete data and unavailability of their charts at the time of data collection. A total of 153 cases and 153 controls with a ratio of 1:1 were included in the study. The mean age at starting treatment was 32 and 36 with a standard deviation of 7 and 10 year for cases & controls respectively. Majorities 82(53.6%) cases and 78(51%) controls were females. Majority 90.2% of cases and 86.3% of controls of the study subjects were orthodox in religion.

Regarding to marital status, 35 (22.9%) of cases and 31 (20.3%) controls were never married and 65 (42.5%) of cases and 47 (30.7%) of controls were married. About half 48 (31.4%) of cases and 50(32.7%) of controls unemployed while 40 (26.1 %) of cases and 30 (19.6%) of controls were daily laborer & most 56(36.6%) cases and 85(55.6%) controls were secondary school and above by their education level at time of HAART initiation. Of the study participants 20.25% have history of substance use. .The socio-Demographic characteristics of the study participants are shown in the table 1 below.

Table 1 .Socio-demographic characteristics among cases and controls at Gondar University Hospital 2015.

Variables	**category**	**VIROLOGIC failure**					
		Controls		cases		Total	
		N	%	N	%	N	%
Age	<35 years	73	47.7%	99	64.7%	172	56.2%
	≥35 years	80	52.3%	54	35.3%	134	43.8%
Sex	Male	74	48.4%	71	46.4%	145	47.4%
	Female	78	51.0%	82	53.6%	160	52.3%
Residence	urban	122	79.7%	116	75.8%	238	77.8%
	rural	31	20.3%	37	24.2%	68	22.2%
Marital status	never married	31	20.3%	35	22.9%	66	21.6%
	married	47	30.7%	65	42.5%	112	36.6%
	others	75	49.0%	53	34.6%	128	41.8%

Educational level	no educated	34	22.2%	42	27.5%	76	24.8%
	primary	34	22.2%	55	35.9%	89	29.1%
	secondary	85	55.6%	56	36.6%	141	46.1%
Religion	Orthodox	132	86.3%	138	90.2%	270	88.2%
	Others	21	13.7%	15	9.8%	36	11.8%
Occupation	Not employed	50	32.7%	48	31.4%	98	32.0%
	Employed	42	27.5%	24	15.7%	66	21.6%
	self employed	31	20.3%	41	26.8%	72	23.5%
	daily laborer	30	19.6%	40	26.1%	70	22.9%

5.2. Clinical characteristics

According to WHO clinical staging majority of participants 121(79.1%) was classified as stage III&IV; at enrolment. Tuberculosis was occurred in 33(26.1%) cases 20(13.1)controls and, 40(26.1%), cases 44(28.8%) controls before and after, starting ART respectively. most of the study participants276 (91.3%) were enrolled to cotri-moxazol prophylaxis therapy when was indicated. The BMI of the study participants were below 16.5kg/m2 in 22(14.4%) cases and 18(11.8%) controls and 28(18.3)of cases and 16(10.5) at the beginning of ART and currently respectively. Most of the study participants 47(31.3%) cases and 78(51%) controls were started their first line ART with AZT based regimen and 86(56.2%) of cases and 90 (58.8%) of controls were on AZT based regimen.D4T based regimen was insignificant (<5) at the time of treatment failure diagnosis in both groups .nearly half of the study participants 47.4%(47.7% of cases and 47.1% of controls has history of change their ART regimen or individual ARV medication. Regarding to adherence half of cases76 (49.7%) and 12(7.8%) controls had history of poor adherence. Most of the study subjects 109(71.2%) of cases and 75(49%) of controls were on HAART for more than 48 months. The clinical data of study subjects is shown in the table below.

Table 2: clinical and ARV medication related information among cases and controls at Gondar University Hospital 2015

Variables	Category	Virologic failure					
		Control		Cases		Total	
		N	%	N	%	N	l %
Baseline BMI	<16.5	18	11.8%	22	14.4%	40	13.1%
	16.5-18.49	49	32.2%	48	31.4%	97	31.7%
	≥18.5	85	55.9%	83	54.2%	168	54.9%
Current BMI	<16.5	16	10.5%	28	18.3%	44	14.4%
	16.5-18.49	28	18.4%	27	17.6%	55	18.0%
	≥18.5	108	71.1%	98	64.1%	206	67.3%
WHO clinical stage	stage I & II	42	27.5%	32	20.9%	74	24.2%
	stage II & IV	111	72.5%	121	79.1%	232	75.8%
ADHERENCECAT	Good	141	92.2%	77	50.3%	218	71.2%
	Poor	12	7.8%	76	49.7%	88	28.8%
History of TB	No TB	89	58.2%	80	52.3%	169	55.2%
	Before ART	20	13.1%	33	21.6%	53	17.3%
	After ART	44	28.8%	40	26.1%	84	27.5%
CPT When indicated	NO	14	9.2%	11	7.2%	25	8.2%
	Yes	139	90.8%	142	92.8%	281	91.8%
First line ART regimen	D4T Based	38	24.8%	64	42.7%	102	33.3%
	AZT Based	78	51.0%	47	31.3%	125	40.8%
	TDF Based	37	24.2%	39	26.0%	76	24.8%
Change of ARV regimen or individual drugs	No	81	52.9%	80	52.3%	161	52.6%
	Yes	72	47.1%	73	47.7%	145	47.4%
ART Regimen at the time of VL test	AZT Based	90	58.8%	86	56.2%	176	57.5%
	TDF Based	63	41.2%	67	43.8%	130	42.5%

Duration with HIV	6-25	28	18.3%	9	5.9%	37	12.1%
	25-48	46	30.1%	30	19.6%	76	24.8%
	≥48	79	51.6%	114	74.5%	193	63.1%
ARTDURATION	6-25	38	24.8%	13	8.5%	51	16.7%
	25-48	40	26.1%	31	20.3%	71	23.2%
	≥48	75	49.0%	109	71.2%	184	60.1%
Substance use	NO	118	77.1%	126	82.3%	244	79.7%
	YES	35	22.9%	27	17.6%	62	20.3%
	Total	153	100%	153	100%	306	100%

Laboratory information

Most of the study participants 120 (78.9%), 73(48.4%) controls and 135 (88.2%), 125(81.7%) cases had baseline and current CD4 count below 200cells/mm3 respectively. Only one- third 36(23.8%) controls and 56(37.8%) of cases of the study participants had current WBC count of<4,000cells/mm3.

Majority 127(84.1%) of controls and 113(75.8%) of cases had current hemoglobin of >112g/dl.

The laboratory information is shown in the table below.

Table 3. Laboratory information among cases and controls at Gondar university Hospital 2015

Variables	**category**	**Virologic failure**					
		Control		Cases		Total	
		N	%	N	%	N	%
current WBC	<4,000c/mm3	36	23.8%	56	37.8%	92	30.8%
	>4,000c/mm3	115	76.2%	92	62.2%	207	69.2%
Current	<12g/dl	24	15.9%	36	24.2%	60	20.0%
Hemoglobin	>12g/dl	127	84.1%	113	75.8%	240	80.0%
Baseline CD4	<200c/mm3	120	78.9%	135	88.2%	255	83.6%
count	>200c/mm3	32	21.1%	18	11.8%	50	16.4%
Current CD4	<200c/mm3	74	48.4%	125	81.7%	199	65.0%
count	>200c/mm3	79	51.6%	28	18.3%	107	35.0%

5.3. Factors associated with HIV virologic treatment failure

In a bivariate analysis with P value < 0.2, age, marital status, education, occupation, adherence, TB Diseases, WHO clinical stage, current BMI, first line ART, duration on ART, duration with HIV, baseline and current CD4 count ,current WBC count, current Hemoglobin level, were associated with antiretroviral virologic treatment failure.

Table 4. Factors associated with ART treatment failure Gondar university hospital2015.

variables	Category	Control NO	%	Cases NO	%	p	COR	95%CI	
								Low	up
Age	<35 years	73	47.7%	99	64.7%	.003	2.009	1.27	3.18
	≥35 years	80	52.3%	54	35.3%	.026	.675		
Marital status	Never married	31	20.3%	35	22.9%	.032			
	Married	47	30.7%	65	42.5%	.516	1.225	.664	2.26
	Others	75	49.0%	53	34.6%	.124	.626	.344	1.14
Educational level	no educated	34	22.2%	42	27.5%	.003			
	Primary	34	22.2%	55	35.9%	.029	1.875	1.07	3.3
	Secondary	85	55.6%	56	36.6%	.001	2.455	1.42	4.23
Occupation	Not employed	50	32.7%	48	31.4%	.055			
	Employed	42	27.5%	24	15.7%	.112	.595	.314	1.13
	self employed	31	20.3%	41	26.8%	.305	1.378	.747	2.54
	daily laborer	30	19.6%	40	26.1%	.297	1.389	.749	2.57
WHO clinical stage	stage I & II	42	27.5%	32	20.9%				
	stage II & IV	111	72.5%	121	79.1%	.183	1.431	.845	2.42
ADHERENCE	Good	141	92.2%	77	50.3%				
	Poor	12	7.8%	76	49.7%	000	12.45	6.26	24.9
History of TB	No TB	89	58.2%	80	52.3%	.150			
	Before ART	20	13.1%	33	21.6%	.060	1.836	.976	3.45
	After ART	44	28.8%	40	26.1%	.966	1.011	.599	1.71
First line ART regimen	D4T Based	38	24.8%	64	42.7%	.000	.358	.208	.614
	AZT Based	78	51.0%	47	31.3%	.128	.626	.342	1.1
	TDF Based	37	24.2%	39	26.0%	.001			

ARTDURATION	6-25	38	24.8%	13	8.5%	.000			
	25-48	40	26.1%	31	20.3%	.041	2.265	1.033	4.968
	≥48	75	49.0%	109	71.2%	.000	4.248	2.120	8.52
Current BMI	<16.5	16	10.5%	28	18.3%	056	1.929	.985	3.78
	16.5-18.49	28	18.4%	27	17.6%	.841	1.063	.586	1.97
	≥18.5	108	71.1%	98	64.1%	.158			
current WBC	<4,000c/mm3	36	23.8%	56	37.8%	.009	1.944	1.18	3.21
	≥4,001c/mm3	115	76.2%	92	62.2%				
Current Hemoglobin	<12g/dl	24	15.9%	36	24.2%	.075	1.686	.948	2.99
	≥12.1g/dl	127	84.1%	113	75.8%				
Baseline CD4 count	<200c/mm3	120	78.9%	135	88.2%	.030	2.000	1.07	3.75
	≥201c/mm3	32	21.1%	18	11.8%				
Current CD4 count	<200c/mm3	74	48.4%	125	81.7%	.000	4.766	2.839	8.0
	≥201c/mm3	79	51.6%	28	18.3%				

Others (Muslim &protestant)

Multivariable analyses

All variables associated with first line treatment failure in the bivariate analysis with P value <0.2 were entered in a multivariable logistic regression analysis. Finally, age, marital status, history of poor adherence, current CD4 count <200 cell/mm3 and duration on ART were independently associated with antiretroviral virologic treatment failure.

Table 5. Independent predictor's of ART virologic treatment failure in Gondar university hospital 2015.

Variables	Category	Control		Cases		P	COR	95%CI		P	AOR	95%CI
		NO	%	NO	%			L	upp			
Age	<35 years	73(47.7%)		99	64.7%	.03	2(1.27-3.18)			.04	2.52(1.33-4.77)	
	≥35 years	80(52.3%)		54	35.3%	.026	.67			1		
Marital status	never married	31	20.3%	35	22.9%	.032				1		
	Married	47	30.7%	65	42.5%	.516	1.23(.66-.26)					
	Others	75	49.0%	53	34.6%	.124	.63(.34-1.14)			.04	2.1(1.10-4.11)	
Educational level	no educated	34	22.2%	42	27.5%	.003						
	Primary	34	22.2%	55	35.9%	.029	1.88	1.07	3.23			
	Secondary	85	55.6%	56	36.6%	.001	2.56	1.42	4.23			
Occupation	Not employed	50	32.7%	48	31.4%	.055						
	Employed	42	27.5%	24	15.7%	.112	.59	.31	1.13			
	self employed	31	20.3%	41	26.8%	.305	1.38	.75	2.54			
	daily laborer	30	19.6%	40	26.1%	.29	1.39	.75	2.57			
WHO clinical stage	stage I & II	42	27.5%	32	20.9%							
	stage II & IV	111	72.5%	121	79.1%	.18	1.43	.84	2.42			
ADHERENCE	Good	141	92.2%	77	50.3%					1		
	Poor	12	7.8%	76	49.7%	000	12.4	6.26	24.9	00	15.8(6.9-36.5)	
History of TB	No TB	89	58.2%	80	52.3%	.150						
	Before ART	20	13.1%	33	21.6%	.060	1.84	.976	3.45			
	After ART	44	28.8%	40	26.1%	.966	1.01	.599	1.71			
First line ART regimen	D4T Based	38	24.8%	64	42.7%	.000	.358	.208	.614			
	AZT Based	78	51.0%	47	31.3%	.128	.626	.342	1.14			
	TDF Based	37	24.2%	39	26.0%	.001						
Duration	6-25	38	24.8%	13	8.5%	.000				1		

on ART	25-48	40	26.1%	31	20.3%	.041	2.26	1.03	4.97	.04	3(1.1-8.4)
	≥48	75	49.0%	109	71.2%	.000	4.25	2.12	8.52	00	6.7(2.7-16.6)
Current	<16.5	16	10.5%	28	18.3%	056	1.92	.98	3.78		
BMI	16.5-18.49	28	18.4%	27	17.6%	.841	1.06	.58	1.97		
	≥18.5	108	71.1%	98	64.1%	.158					
current	<4,000c/mm3	36	23.8%	56	37.8%	.01	1.94	1.18	3.20		
WBC	≥4,000c/mm3	115	76.2%	92	62.2%						
Current	<12g/dl	24	15.9%	36	24.2%	.075	1.68	.95	2.99		
Hemoglobin	≥12g/dl	127	84.1%	113	75.8%						
Baseline	<200c/mm3	120	78.9%	135	88.2%	.030	2.00	1.07	3.75		
CD4 count	≥200c/mm3	32	21.1%	18	11.8%						
Current	<200c/mm3	74	48.4%	125	81.7%	.000	4.77	2.84	8.00	00	9.03(4.4-18.5)
CD4 count	≥200c/mm3	79	51.6%	28	18.3%						

COR (crud odds ratio), AOR (adjusted for other variables), CI (confidence interval)

6. Discussion

The study has provided an opportunity to find out determinants of first line antiretroviral virologic treatment failure which is globally a very serious challenge for antiretroviral treatment program. In the study, risk factors antiretroviral virologic treatment failure was determined in adult HIV-infected population in Gondar university Hospital. Among this factors recent CD4 count <200 cell/µl was significantly associated with antiretroviral virologic treatment failure. Patients with recent CD4 count below 200cell/µl failed9 times than that of with CD4 count of>200 cell/µl with 95% CI (4.4-18.5) The result is consistent with other study done in central Cameron yawn die; a recent CD4 count less than 200 cells per microliter increased the odds of failure more than 7.4 folds this could be presence of drug resistant virus at initiation of ART [16].

Patients who have history of poor adherence were failed15.8 times when compared with those who have no history of poor adherence with 95% (CI 6.9 to 36.5). The result is comparable with a study done in Cameron; the odds of treatment failure were more than 12.8 folds for Patients who had poor adherence [16]. The very high risk of first line antiretroviral treatment failure in the study in patients with unplanned interruptions to therapy raises a concern that the failure to stagger ART cessation may be contributing to subsequent treatment failure which is consistent with several previous reports [5, 8].Another risk factors determined were age <35years with adjusted odds ratio of 2.52 more likely to develop ART virologic treatment failure when compared with age greeter than 35 years with (CI-1.33 to 4.77),this is similar with study done in South Africa and France shows that age less than 30 years was associated with ART virologic treatment failure(7,15).this is probably due to loose of hop and stress which leads to poor adherence to ART medication. Another factor which significantly associated was long duration on ART. The odds of treatment failure in those for 25-48 months of treatment was 3 times when compared with less than 25 months of treatment with CI(1.1-8.4) and 6 ,7times more likely in those with greater than 48 months of treatment with CI (2.7-16.6). This study is similar with studies in Lesotho (16).This is probably due to drug resistance and other opportunistic infections. The other associated factor in this study is marital status those who separated, divorced, and widowed were more likely to develop treatment failure 2.1times when compared with never married with CI (1.10-4.11).

These in line with a study done in Gabon divorced were at high risk of ART virologic treatment failure (14). This is probably due to lack of social support and instability of family.

Strengths

- It is done in better and well organized ART clinic where medical records and data is well managed.
- The data was collected by appropriate trained nurse who know the terminologies across the data collection process.

Limitations

Poorly recorded clinical information during the follow up period affects to explore additional determinant factors.

7. Conclusion

Majorities 82(53.6%) cases and 78(51%) controls were females. Majority 90.2% of cases and 86.3% of controls of the study subjects were orthodox in religion. Regarding to marital status, 35 (22.9%) of cases and 31 (20.3%) controls were never married and 65 (42.5%) of cases and 47 (30.7%) of controls were married. According to WHO clinical staging majority of participants 121(79.1%) was classified as stage III&IV; at enrolment. Tuberculosis was occurred in 33 (26.1%) cases 20(13.1) controls and, 40(26.1%), cases 44(28.8%) controls before and after, starting ART respectively

Most of the study participants120 (78.9%), 73(48.4%) controls and135 (88.2%), 125(81.7%) cases had baseline and current CD4 count below 200cells/mm3 respectively

The independent predictors for antiretroviral virologic treatment failure were; recent cd4 count lower than 200 cell/µl, history of poor adherence for ART, age<35 years, marital status and long duration on ART. Based on the findings of the study the following recommendation is forwarded.

8. Recommendations

Health professionals should pay attention to give strict adherence counseling for patients and monitoring routine CD4 count to monitor ART treatment response and to diagnose early treatment failure.

Patients should take their medication as prescribed and consult their clinician when needed.

Government body should monitor and evaluate recordings system and improve recording system.

Finally further study on patients follow up and quality of care should be done in the future.

9. References

1. Paul A. medical managmant of HIV infection. USA: knowledge soure solution,LLC; 2009-2010. Available from: WWW.mmhiv.org.
2. Organization WH. Global update on the health sector response to HIV, 2014. 2014.
3. Aberg JA, Gallant JE, Khalil G. Ghanem, Emmanuel P, Zingman BS, Horberg aMA. Primary Care Guidelines for the Management ofPersons Infected With HIV: 2013 Update by theHIV Medicine Association of the InfectiousDiseases Society of America. 2013.
4. Chang LW, Harris J, Humphreys EH. Optimal monitoring strategies for guiding when to switch first-line antiretroviral therapy regimens for treatment failure in adults and adolescents living with HIV in low-resource settings. The Cochrane Library. 2010.
5. Harries AD, Zachariah R, van Oosterhout JJ, Reid SD, Hosseinipour MC, Arendt V, et al. Diagnosis and management of antiretroviral-therapy failure in resource-limited settings in sub-Saharan Africa: challenges and perspectives. The Lancet infectious diseases. 2010;10(1):60-5.
6. Jordan M, Bennett D, Bertagnolio S, Gilks C, Sutherland D. World Health Organization surveys to monitor HIV drug resistance prevention and associated factors in sentinel antiretroviral treatment sites. who. 2012;13:15.
7. Organization WH. March 2014 supplement to the 2013 consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection: recommendations for a public health approach. 2014.
8. ethiopian national ART Guidline. In: health mo, editor. Addis-Abbaba2014. p. 71.
9. Renaud-Théry F, Duncombe C, Kerr S, Thierry S, Perriens J, editors. Adult antiretroviral therapy in resource limited settings: a systematic review of first-line failure and attrition rates. 17th Conference on Retroviruses and Opportunistic Infections, San Francisco; 2010.
10. Labhardt ND, Bader J, Ramoeletsi M, Kamele M, Lejone TI, Cheleboi M, et al. Clinical and socio-demographic predictors for virologic failure in rural Southern Africa: preliminary findings from CART-1. J Int AIDS Soc. 2014;17(4 Suppl 3):19666. PubMed PMID: 25397416. Pubmed Central PMCID: PMC4225252. Epub 2014/11/15. eng.
11. Liegeois F, Vella C, Eymard-Duvernay S, Sica J, Makosso L, Mouinga-Ondeme A, et al. Virological failure rates and HIV-1 drug resistance patterns in patients on first-line

antiretroviral treatment in semirural and rural Gabon. J Int AIDS Soc. 2012;15(2):17985. PubMed PMID: 23199801. Pubmed Central PMCID: PMC3510650. Epub 2012/12/04. eng.

12. Paris D, Ledergerber B, Weber R, Jost J, Flepp M, Opravil M, et al. Incidence and Predictors of Virologic Failure of Antiretroviral Triple-Drug Therapy in a Community-Based Cohort. AIDS research and human retroviruses. 1999 1999/12/10;15(18):1631-8.
13. Paredes R, Lalama C, Ribaudo H, Schackman B, Shikuma C, Giguel F, et al. Pre-existing minority drug-resistant HIV-1 variants, adherence, and risk of antiretroviral treatment failure. J Infect Dis. 2010 Mar;201(5):662-71. PubMed PMID: 20102271. Pubmed Central PMCID: PMC2825289. Epub 2010/01/28. eng.
14. Borroto-E, Waters J, Bae A, Harris J, Hinkle J, Quinn J, et al. Baseline genotype as a predictor of virological failure to emtricitabine or stavudine in combination with didanosine and efavirenz. AIDS research and human retroviruses. 2007;23(8):988-95.
15. Rougemont M, Stoll BE, Elia N, Ngang P. Antiretroviral treatment adherence and its determinants in Sub-Saharan Africa: a prospective study at Yaounde Central Hospital, Cameroon. AIDS Res Ther. 2009;6(21):6405-6.
16. Chkhartishvili N, Sharvadze L, Dvali N, Karchava M, Rukhadze N, Lomtadze M, et al. Virologic outcomes of second-line antiretroviral therapy in Eastern European country of Georgia. AIDS Res Ther. 2014;11:18. PubMed PMID: 25035708. Pubmed Central PMCID: PMC4102034. Epub 2014/07/19. eng.
17. Wolbers M, Opravil M, von Wyl V, Hirschel B, Furrer H, Cavassini M, et al. Predictors of optimal viral suppression in patients switched to abacavir, lamivudine, and zidovudine: the Swiss HIV Cohort Study. AIDS. 2007 Oct 18;21(16):2201-7. PubMed PMID: 18090047. Epub 2007/12/20. eng.
18. Teshome W, Assefa A. Predictors of immunological failure of antiretroviral therapy among HIV infected patients in Ethiopia: a matched case-control study. PLoS One. 2014;9(12):e115125. PubMed PMID: 25536416. Pubmed Central PMCID: PMC4275231. Epub 2014/12/24. eng.
19. Rutherford GW, Anglemyer A, Easterbrook PJ, Horvath T, Vitoria M, Penazzato M, et al. Predicting treatment failure in adults and children on antiretroviral therapy: a systematic review of the performance characteristics of the 2010 WHO immunologic and clinical criteria for virologic failure. AIDS. 2014;28(Suppl 2):S161-S9.

10. Annexes

Annex I: Information sheet

Title of the research project: determinants of HIV virologic treatment failure at Gondar university Hospital, North west Ethiopia 2015

Principal Investigator: Belete Bayu

Advisors:

Mr. Amare Tariku (MSc)

Dr. Abera Balcha

Name of the organization: University of Gondar, Collage of Medicine and Health Sciences, Department of internal medicine

Sponsor: Amhara regional health bureau

Introduction

This checklist is prepared for the collection of socio-demographic, HIV care and antiretroviral treatment and laboratory related information to determine predictors of ART virologic failure among Adult HIV infected clients at University of Gondar hospital. All this information will be retrieved from the clients ART and pre ART registration book and from individual patient card without mentioning the name of the clients. This information will be collected by health care providers working in the ART clinic at Gondar university Hospital.

Annex II: Questionnaire

General information

1	MRN/HMISNO:		
2	UAN		
3	Appointment date	1—---------- 2----------	
4	Telephone number-or recent contact address to tack patient		

Socio-demographic information

1	Age		
2	Sex	Male Female	1 2
3	Residence write specific location residence information	_____ _____	
4	Marital status	Never married Married including de facto Separated Divorced Widow/widower	1 2 3 4 5
5	Level of education	No education Primary Secondary Tertiary	1 2 3 4
6	Religion	Orthodox Muslim Protestant Catholic Other specify----------	1 2 3 4 5

7	Occupation Note : a house wife with no income will fall under unemployed see HIV/ART intake form	Not employed Civil servant/govt employee Farmer Merchant/retailer Daily laborer/migrant worker Other specify----------	1 2 3 4 5 6

HIV care and anti-retroviral treatment related information

1	Write duration (in months)since time of HIV TEST confirmed Note: please refer ART intake & follow-up form	1.date of HIV test (dd/mm/yyyy ____/_____/_____ 2. months_____	
2	Write duration (in months)since time of eligibility for ART Note: please refer ART intake & follow-up form	1.Date of eligibility for ART _____/_____/_____ 2.months_____	
3	**Duration on ART** in months Note: please refer ART intake & follow-up form	1. Date of eligibility for ART ____/_____/_____ 2. Months_____	
4	Ever exposed for any ARV drugs before initiation of ART. Note :consider all exposers to ART before ART is started based on national ART guideline	Yes No	1 2
5	If exposed which ARV drug or ART regimen Note: please refer ART intake & follow-up form or PMTCT code on ART follow-up card	A-combination ART (HAART) but stopped B- PMTCT prophylaxis (sd-NVP, AZT, or combination ARV) C-Prophylaxis other than for PMTCT (PEP, prep) D-Others specify_____	

6	Base line BMI. Note: see at “zero” month	Kilogram/meter square_____	
7	Current BMI. note: write BMI at last visit	Kilogram/meter square_____	
8	Ever pregnant while taking ART	1-yes 2-no 3-note applicable(male sex)	1 2 3
9	Current functional status Note: if client has different functional assessment result /category please write the most recent one	W-working A –Ambulatory B-Bed-ridden	1 2 3
10	WHO Clinical stage of diseases at enrolment Note: write WHO clinical stage of AIDS at start of ART as written on the ART intake or follow up form	Stage I Stage II Stage III Stage IV	1 2 3 4
11	Screened for tuberculosis in the last 6 months(i.e. while taking ART)	Yes, positive Yes, negative for TB	1 2
12	If screened for TB is hey/she on treatment or prophylaxis? note: applicable to the last six months (i.e. while taking ART)	On treatment for TB (TB Rx) On INH or prophylaxis for TB NO prophylaxis or treatment	1 2 3
13	Ever enrolled to cotrimoxazole (TMP-SMX) Preventive prophylaxis in the last 6 months Note: make “yes” if drug doses or duration is documented .if started in the last 6 months but stopped mark yes	Yes No	1 2
14	Current adherence to ART Status Note: if client has different adherence assessment results /category please write the most recent one (among those documented in the last 6 months)	G-Good F-Fair P-Poor	1 2 3

15	Write the original first line ART initiated at zero month)enrolment to ART program Note: write correct and original first line ART. For transfer ins (TI) write the combination of ART initiated for the first time at referring clinic (here please circle on exact same regimen the patient has been started the first time check only one. If you can't identify a particular drug from the below drug list ,please make on "other " and write the name of every drug on the blank space .otherwise, please do not write codes or abbreviations	First line ART 1a=d4T+3TC+NVP 1b=d4T-3TC+EFV 1C=ZDV+3TC+NVP 1d=ZDV+3TC+EFV 1e=TDF+3TC+EFV 1f=TDF+3TC+NVP Alternative or second line ART 2a=ABC+ddI+LPV/r 2b=TDF+3TC+LPV/r 2c=ZDV+3TC+LPV/r 2d=ZDV+ABC+LPV/r Others specify_____	 1 2 3 4 5 6 7 8 9 10 11
16	Any change or switch of ARV regimen or individual drugs Note: change in manufacturer or brand of a drug is not considered switch or change.	Yes, regimen /drug is changed NO (it is the same regimen & drug)	1 2
17	Write the current ART regimen (Here please circle on exact same regimen the patient is currently taking or has been taking in the previous three months .check only one. If you can't identify a particular drug from the below drug list ,please make on "other " and write the name of every drug on the blank space .otherwise, please do not write codes or abbreviations	First line ART 1a=d4T+3TC+NVP 1b=d4T-3TC+EFV 1C=ZDV+3TC+NVP 1d=ZDV+3TC+EFV 1e=TDF+3TC+EFV 1f=TDF+3TC+NVP Alternative or second line ART 2a=ABC+ddI+LPV/r 2b=TDF+3TC+LPV/r 2c=ZDV+3TC+LPV/r 2d=ZDV+ABC+LPV/r Others specify_____	 1 2 3 4 5 6 7 8 9 10 11

18	Write all reasons for change or switch of ARV regimen or individual drug. first all option /choices Refer codes or "reason for change "on the back of national ART follow-up chart or intake form and progress sheet. Important :consider recent changes of regimen due to staved in phase-out program as toxicity of drug" Do not guess reasons; consult consultant or ART physician.	<u>Toxicity of drug</u> Yes No <u>Pregnancy</u> Yes No <u>Drug stock out</u> Yes No Other please specify_____	 1 2 1 2 1 2 3
19	Any opportunistic diseases **<u>in the last six months</u>** while on ART Note: please document new or recurrent WHO stage IV conditions and the following stage III conditions pulmonary TB sever bacterial infection (source national ART guideline) Note: please use abbreviations that match the list on the back of national ART follow up form.	PCP, pneumocystiscarini pneumonia BP ,sever bacterial pneumonia CT, CNS toxoplasmosis CM, Cryptococcal meningitis PTB, pulmonary tuberculosis ETB, exta-plumonary TB, or disseminated TB(other than lymph nod TB) None or OI is not recorded Other (specify)	1 2 3 4 5 6 7 8

LABORATORY INFORMATION

1	**<u>Base line WBC</u>** count before /at initiation of ART Note : write the test result closes to ART initiation date (+-30 days)	WBC_____/mm3 Note available	 000
2	**<u>Current WBC count</u>** Note: when there are different test results take the most recent in the last six months as current	WBC_____/mm3	

3	**Base line hemoglobin** result before /at initiation of ART Note: write the test result close to ART initiation date (+-30 days) Note: when **hemoglobin** is not available fill in **hematocrit**	Hgb_____mg/dl Hct_____% Note available on record	 000
4	Current hemoglobin Note: take the most recent hemoglobin result in the last 6 month as current	Hgb_____mg/dl Hct_____%	
5	Base line cd4 test **result** before start of ART Note: write the test result closest to ART initiation date(+-30 days)	Cd4_____/mm3 Not available on record	 000
6	**Peak cd4 tests while on ART** Note: record the highest cd4 count ever recorded. If different from the one recorded on TFSS, write the recent result.	_____/mm3	
7	**Current cd4 test result** Note: record the recent result (<-6months).if different from the one recorded on TFSS write the recent result.	Cd4_____/mm3	
8	Base line viral load test Note: record any viral load test done prior to 90 days (three months)	_____c/ml Note available on record	 000
9	Current viral load test Note: record the most recent HIV viral load result <-90) days and the test date from records (charts or laboratory register)	VL_____C/ml Date(DD/MM/YYYY) _____/_____/_____	

Complete forms will be submitted to hospital HIV case team leader or CEO

Audit completed by_____signature_____

Audit supervised by_____signature_____

Annex III: Student Declaration

I, the under signed, senior MSC student declare that this thesis proposal is my original work in partial fulfillment of the requirement for the master's degree in Clinical tropical infectious diseases and HIV medicine.

Name: _____

Signature: _____

Place of submission: Department of internal medicine, College of Medicine and Health Science, University of Gondar.

Date of submission_____

This thesis proposal work has been submitted for examination with our approval as University Advisors.

Advisors

Name	signature
1._____	_____
2._____	_____

Annex IV: Assurance of the investigator

The under signed agree to accept responsibility for the scientific, ethical and technical conduct of the research project and for provision of required process report as pre terms and conditions of research and publication office of the University of Gondar.

Name of the student _____

Date_____ signature_____

Approval of the advisors

Advisors

Name	Signature	Date
1._____	_____	_____
2._____	_____	_____